Wheat Germ Oil and Its Effects on a Liquid Larval Rearing Diet for Oriental Fruit Flies (Diptera: Tephritidae)

CHIOU LING CHANG[1] AND ROGER I. VARGAS[2]

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ABSTRACT Wheat germ oil was added to a larval liquid diet for rearing *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) to optimize fruit fly quality. Effects of various concentrations of wheat germ oil at 0.04, 0.07, 0.15, 0.30, and 0.66% and their possible mode of action were evaluated. Results suggest that addition of wheat germ oil does not affect pupal weight, larval developmental period, adult emergence, mating ability, or peak time for egg production. But there was a significant increase in pupal recovery, percentage of adult fliers, egg production, or egg hatch for larvae fed the diet with wheat germ oil compared with those reared on the liquid diet without wheat germ oil. The increase in egg hatch and fliers was dose dependent. Therefore, addition of wheat germ oil to fruit fly rearing diet is a novel way to improve fruit fly quality, especially in egg hatch, fliers, egg production, and pupal recovery.

KEY WORDS wheat germ oil, liquid diet, *Bactrocera dorsalis*, fruit fly rearing

Wheat germ oil (WGO) is known to be one of nature's richest sources of polyunsaturates, vitamin E, and octacosanol, and it has been used for decades for various reasons. WGO has been reported as a diet supplement for farm animals, racehorses, pets, and minks (Barnes 1982, 1983); as an additive in health products to change plasma and liver cholesterol (Lairon et al. 1987, Kahlon et al. 1988) or to promote physical endurance and retard aging (Kahlon 1989); as a breeding aid for livestock and poultry (Levin 1945); as a fertility agent in cows (Kahlon 1989); as an antioxidant as a natural source of α-tocopherols (Mostafa 1982, Krings and Berger 2001); as a potential biological control agent for dermestid beetle *Trogoderma glabrum* (Herbst) larvae due to their aggregation response (Nara et al. 1981), and as an aid for oxygen use and reproduction in rats to reduce embryonic mortality and promote viability (Dukelow 1967).

WGO improves fertility in vertebrates, such as golden hamsters (Soderwall and Smith 1962), White Leghorn chicks (Levin 1963), sheep (Dukelow and Matalamaki 1963), cows (Marion 1962; Dukelow 1966; 1967), and humans (Currie 1937, Silbernagel 1947, Dukelow 1967, Jager 1975). There are a few reports that WGO or wheat germ improves fertility, growth, and survival in insects (McFarlane and Alli 1985, Vargas et al. 1994). *Bactrocera dorsalis*, (Hendel) (Diptera: Terphritidae), the oriental fruit fly, whose larvae were reared on a liquid larval rearing diet (Chang et al. 2006), resulted in low fertility in the early stage of liquid diet development. Chang's diet was based on Tanaka's standard mill feed diet for fruit fly rearing (Tanaka et al. 1969). We speculate that dietary fatty acids in the liquid diet formulation may be a crucial deficient factor because mill feed, which serves as a bulking agent in Tanaka's diet, was replaced with the inert supporting matrix, sponge cloth. This is because mill feed diet contains 9.85 times more total fatty acids (including unsaturated and saturated) than those in a liquid diet (Chang et al. 2004). In WGO, unsaturated fatty acids account for $\approx$80% and saturated fatty acids 20% of the total fatty acid content (Nelson et al. 1963, Lercker et al. 1977, Barnes 1983, Saker et al. 1986). In addition, WGO is rich in vitamin E. Its tocopherol content (both α- and β-form) is reported to range from 71 to 133 mg/100 g (Kahlon 1989). The effects of WGO on adult quality for fruit fly larvae reared on a newly developed low waste liquid diet were investigated.

Materials and Methods

Insects. One-hour-old eggs of the oriental fruit fly were provided by the fruit fly rearing laboratory at the Agricultural Research Service, Pacific Basin Agricultural Research Center in Honolulu, HI. The colony has been reared on a wheat-based mill feed diet for >360 generations (Table 1) (Tanaka et al. 1969). The adult colony room was maintained at 25°C, 65% RH, and a photoperiod of 12:12 (L:D) h.

Diet Preparation. Six diets were prepared: one according to the standard recipe without wheat germ oil (Table 1), and the other five diets with the addition of either 0.04, 0.07, 0.15, 0.30, or 0.66% (vol:vol) wheat germ oil (ICN Biomedicals Inc., Aurora, OH). Diet

[1] Corresponding author: U.S. Pacific Basin Agricultural Research Center, USDA–ARS, 2727 Woodlawn Dr., Honolulu, HI 96822 (e-mail: schang@pbarc.ars.usda.gov).

[2] U.S. Pacific Basin Agricultural Research Center, USDA–ARS, P.O. Box 4459, Hilo, HI 96720.

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Table 1. Liquid diet composition (in grams) of *B. dorsalis*

Ingredient	Amount (g)	Specific gravity[a]	%
Sugar	12.18	1.587	8.99
Brewer's yeast	20.40	1.015	15.05
Nipagen	0.20	1.1–1.2	0.15
Sodium benzoate	0.20	1.116–1.120	0.15
Citric acid	2.31	1.54	1.70
Water	100.00	1.0	73.80
Wheat germ oil	0.20	0.93	0.15

[a] Specific gravities are shown in here for unit conversion, especially for wheat germ oil, from grams to milliliters or percentage.

ingredients were blended using a commercial Electronic blender (Smoothie Plus 600, Back to Basics Products, Inc., Costco, Honolulu, HI) for ≈2 min on a low setting. These diets were blended for an additional 15 s to ensure uniform distribution of the oil after addition of WGO. Addition of wheat germ oil did not affect the 3.5 pH.

Tray Preparation. Sponge sets (large: 30 by 26 cm; small: 10 by 15 cm) were rinsed in cold distilled water and wrung dry. Lids of pupation fiberglass boxes (50 by 32 by 2.5 cm) (referred to as green tray later) and plastic grids (37 by 28 cm) were washed, soaked for 10 min in a 10% bleach solution, and rinsed in distilled water at least three times. A piece of grid was placed on the bottom of each lid, and a large sponge was placed on top. Liquid diet (1250 ml) was poured over the sponge and into the tray. A small sponge with 2.5 ml of eggs was placed on the top of the large sponge and rinsed with distilled water to spread the eggs. After 4 d, trays were placed in a separate under-bed plastic container (81 by 41 by 13 cm; Iris USA, Inc., Pleasant Prairie, WI) with water in the bottom and a screened cover. The collection water was sieved daily after the larvae began to leave the diet and fall (pop) into the water at day 6. Collected larvae were placed into vermiculite to pupate. Pupae were sifted and weighed in four groups of 100 each at 2 d after collection. The diet in the tray was discarded only after the majority of the larvae had popped (usually 14 d after egg seeding).

Determination of WGO Concentration That Enhanced Fruit Fly Performance. A 0.15% (vol:vol) WGO (2 ml) was added into standard diet as a pilot test to ensure WGO would increase egg hatch and/or have other beneficial effects. Four additional concentrations of WGO (0.04, 0.07, 0.32, and 0.66%) were included in tests to identify the best concentration for cost-effective mass rearing. Liquid diet without WGO was used as a control. All pHs in diets were adjusted to 3.5 by using citric acid. The concentration that produced the highest quality insects (a combination of high percentage of egg hatch, egg production, adult fliers, and pupal recovery) would be selected to optimize the liquid diet.

Identification of Components in WGO and Their Potential Roles in Fruit Fly Performance. Various concentrations (0.15, 0.30, and 0.66% [vol:vol]) of linolenic acid, linoleic acid, vitamin E, and their combinations were substituted for WGO to measure the effects of these components on fruit fly quality. In this study, a preliminary test also was also to identify whether linolenic acid, linoleic acid, or vitamin E alone would enhance hatch of larvae from eggs.

Calculation of Quality Control Parameters. *Pupal Weight.* Eight samples of 100 pupae from each larval recovery were counted and placed into a small sauce cup, and the mean weight (MW) was determined. The largest larval recovery was selected for determination of pupal weight 2 d after collection. Pupal weight was expressed as milligrams per pupa.

Pupal Recovery. To estimate the average number of eggs hatched/100 eggs (H), four sets of 100 eggs each were spread on blotter paper, incubated in petri dishes, and the numbers of hatched larvae were counted 4 d later and averaged. Number of pupae produced from each larval recovery was calculated as total weight of pupae collected from each larval recovery (TW) divided by mean weight (MW). Total number of pupae produced was the sum of each individual recovery. The following formula was used to estimate percentage of pupal production per recovery (R): $R = P/(E \times 15{,}000 \times H/100)$, where P is total number of pupae produced, E is milliliters of eggs seeded, H is average number of eggs hatched, and 1 ml of eggs is estimated as 15,000 eggs.

Developmental Period. Each larval recovery was multiplied by its developmental age (day), and the sum from all pop days was divided by the total pupal production to obtain the peak developmental period (day). This method was derived from Chang et al. (2006).

Adult Fliers. Four samples of 100 pupae inside sauce cups were selected from the largest larval recovery and each placed inside a petri dishes. A 20-cm-long tube of black plastic pipe with a diameter of 8.5 cm was placed over the dish. The tubes were placed in a lighted flight cage (120 by 120 by 60 cm^3) and left until all of the flies had emerged and died, usually 24 d after egg seeding. Flies were placed into one of the following five categories: unemerged, partially emerged (part of adult stick to the puparium), deformed wings (emerged but have deformed wings) and nonflying flies (flies that seem normal but are not capable of flying), and flies capable of flying, and each categories were tallied. Adult emergence was calculated as 1) the total initial number of pupae (100) minus the average percentage of unemerged pupae or 2) total number of flier, nonflier, and deformed wing. Total flying flies was calculated as the initial number of pupae (100) minus total number of nonflying flies, partial emerged, unemerged, and deformed wing.

Egg Production/F_1 Egg Hatch. Twenty grams of pupae was placed in paper bags (6.35 by 10.16 by 7.62 cm). The bags were placed inside egging cages (27 by 25 by 24 cm) with a food mixture of 3:1 (sugar:yeast hydrolysate) (U.S. Biochemical, Cleveland, OH). A covered plastic container of water with a cotton dental wick was added to each cage on the day that the first flies emerged. Beginning 11 d after the flies emerged, eggs were collected and volumes were recorded for each cage for seven consecutive days. Egg hatch sam-

ples—four sets of 100 eggs each from the first egg collection day—were taken from each individual cage. The number of unhatched eggs was recorded 4d later. The percentage of egg hatch was determined by subtracting the sum of unhatched eggs from 100 (Vargas et al. 1994).

The relationship between adult fliers, egg production per female per day, and egg hatch from different concentrations of WGO was determined with regression analysis to identify the effect of these three parameters (SAS Institute 2002).

Peak Egging Period. The number of eggs collected daily was multiplied by its age (day) and tallied. The sum from all collection days was divided by the total eggs collected to obtain the peak egging period (day) (Chang et al. 2006).

Adult Emergence/Sex Ratio. On the same day that the 20-g samples were weighed, four samples of 100 pupae each were counted and placed inside screened plastic containers. These were scored for sex and adult emergence. The data generated were used to estimate the number of pupae, sex ratio (% female) and adult emergence rate within the egging cages.

Mating. One hundred flies of a single sex were aspirated 1 d after emergence into vented 1-liter cups. Flies were provided with water and the standard adult sugar/yeast food mixture. The two sexes were kept isolated in different rooms after sexing until needed at 11 d of age. The mating tests were done in the evening, starting at ≈6:00 p.m. because the oriental fruit fly is known to mate at dusk (Roan et al. 1954, Arakaki et al. 1984). Both males and females were released into clear acrylic cages (30 by 30 by 40 cm), and the lights were turned off. Any dead flies found in the cups were replaced. The tests were run for at least 2 h. Mating pairs were removed from the cages, and tallies of removed pairs were recorded every 30 min.

Statistics. At least four batches of insects were used to replicate for each treatment. Three to four subsamples were taken for each treatment during each test period. Data were statistically analyzed using SAS version 9.1. Differences in diet batch/treatment data were analyzed by one-way analysis of variance (ANOVA) (ANOVA or GLM; $\alpha = 0.05$) followed post hoc by Tukey's honestly significant difference (HSD) test for group mean comparisons. Linear regressions were computed to estimate the correlations between tested concentrations and each of the quality control parameters (SAS Institute 2002).

Results and Discussion

Determination of WGO Concentration That Enhanced Fruit Fly Performance. The hatch of larvae from eggs produced by females reared on the original liquid diet formulation (without wheat germ oil) was very low (35%) (Chang et al. 2006). Based on Canadian and U.S. feed composition tables, Brewer's yeast (main ingredient in Chang's liquid diet) is 3 times more nutritious than mill feed diet in vitamins and proteins, but yeast contains less total fatty acids (1.05%) than mill feed (7.89%). Therefore, addition of wheat germ oil to the standard liquid diet formulation was expected to increase fertility, at least to a comparable level of fertility of flies fed on a mill feed diet.

In a pilot test, 0.15% (vol:vol) WGO was first added to the standard diet (WGO free), resulting in an increase from 31.21 to 66.86% egg hatch (Table 2) compared with those from control diet (81%). Other concentrations of 0.04, 0.07, 0.30, and 0.66% WGO were then tested to seek cost-effective concentrations while maintaining a high-quality fruit fly. For larvae reared on WGO-fortified diets, pupal recovery, percentage of egg hatch, adult fliers, and egg production were significantly affected (Table 2) ($P < 0.05$). Larval duration, pupal weight, adult emergence, sex ratio, mating, and peak egging period calculated for larvae reared on WGO fortified diets were not significantly different from those reared on the standard liquid diet without WGO (Table 2) ($P < 0.05$). Larvae reared on the diet with WGO produced a significant increase in pupal recovery for all tested concentrations, although it was not dose dependent ($r^2 = 0.20$) ($P > 0.05$). These results suggest that there is an increase in production as long as the diet is WGO fortified, even with as low as 0.04% (vol:vol) in the diet. Percentage of egg hatch was significantly increased with the addition of WGO in the diet. This influence is WGO dose dependent ($r^2 = 0.76$). Adult fliers were significantly higher for adults that were reared as larvae on 0.15% or above of WGO fortified diet ($r^2 = 0.78$) ($P < 0.05$). Adults that were reared as larvae on diets with <0.15% WGO were not significantly different in percentage of fliers from those of standard liquid diet-fed flies. These results coincide with the finding of Franenkel and Blewett (1946) and McFarlane and Alli (1985). Fraenkel and Blewett (1946) found that the larvae of the moths belonging to the genus *Ephestia*, *E. kuehniella* Zeller *E. elutella* (Hübner), and *E. cautella* (Walker) grow well on artificial diets that also contain wheat germ oil. In the absence of wheat germ oil growth is slow, mortality is high, and moths fail to emerge from the pupae. They also found that with suboptimal quantities of wheat germ oil moths emerge with the wings lacking in scales. McFarlane and Alli (1985) demonstrated the incorporation of WGO into larval diets of the house cricket, *Acheta domesticus* (L.), has improved survival and growth.

Egg production from *B. dorsalis* females that were reared on WGO-fortified diet as larvae was more than for those reared on a WGO-free diet (Table 2). Egg production for adults that fed on 0.04 and 0.07% WGO-fortified diet as larvae was higher than for those reared on WGO-free diet, but it was significantly lower than for those larvae reared on a WGO-fortified diet with 0.15% or higher concentrations, although over all doses it is not dose dependent ($r^2 = 0.37$) (Table 2).

Wheat germ oil serves as a fertility agent in humans and in many other animals, but very few reports of such an effect as published on insects. Soderwall and Smith (1962) reported that golden hamsters fed WGO throughout pregnancy displayed a higher fertility rate. Levin (1963) reported that topical application of WGO octacosanol to immature White Leghorn chicks

Table 2. Comparison of performance of *B. dorsalis* on diet without WGO and diet fortified with 0.04, 0.07, 0.15, 0.30, and 0.66% of WGO

Parameter	WGO (%)						Significance (PROC ANOVA)
	0	0.04	0.07	0.15	0.30	0.66	
Pupal recovery (%)	34.94 ± 4.39b	49.85 ± 3.28a	54.66 ± 1.96a	51.13 ± 2.36a	53.80 ± 4.25a	52.76 ± 2.23a	$F = 5.06$; df = 5, 22; $P = 0.0051$
Developmental period (d)	9.87 ± 0.24a	9.64 ± 0.27a	9.49 ± 0.28a	9.45 ± 0.18a	9.28 ± 0.24a	10.21 ± 0.39a	$F = 1.46$; df = 5, 22; $P = 0.2554$
Pupal wt (mg)	10.81 ± 0.16a	10.71 ± 0.18a	10.55 ± 0.21a	11.00 ± 0.13a	10.70 ± 0.20a	10.58 ± 0.55b	$F = 0.72$; df = 5, 22; $P = 0.5910$
Sex ratio (female/male)	0.95 ± 0.03a		1.02 ± 0.02a				$F = 3.68$; df = 1, 7; $P = 0.1034$
% adult emergence	97.56 ± 0.81a	98.44 ± 0.41a	98.38 ± 0.22a	98.50 ± 0.35a	98.69 ± 0.73a	97.50 ± 0.42a	$F = 1.62$; df = 5, 87; $P = 0.1632$
Flier (%)	56.57 ± 5.38b	67.41 ± 5.25ab	67.58 ± 4.01ab	77.27 ± 5.18a	77.23 ± 6.12a	86.83 ± 1.40a	$F = 5.57$; df = 5, 87; $P = 0.0002$
Mating (%)	88.92 ± 1.62a			89.87 ± 1.97a		90.21 ± 1.17a	$F = 0.17$; df = 2, 17; $P = 0.8443$
Eggs/female/d	24.47 ± 2.20d	36.21 ± 1.41c	38.29 ± 1.33bc	45.49 ± 0.99a	47.36 ± 1.53a	43.58 ± 1.87ab	$F = 26.44$; df = 5, 42; $P < 0.0001$
Egging period (d)	13.30 ± 0.07b	13.43 ± 0.10b	13.42 ± 0.05b	13.45 ± 0.05b	13.54 ± 0.05ab	13.81 ± 0.08a	$F = 6.55$; df = 5, 42; $P = 0.0002$
Egg hatch (%)	31.21 ± 3.12c	37.64 ± 4.85c	42.18 ± 3.34c	66.86 ± 1.95b	78.86 ± 1.43ab	86.81 ± 0.97a	$F = 68.11$; df = 5, 42; $P < 0.0001$

Within a row, means followed by the same letter are not significantly different ($\alpha = 0.05$; ANOVA test, Tukey's HSD was used for mean separation).

increased comb growth. Dukelow and Matalamaki (1963) administered WGO to ewes (sheep) for 3 wk and found a significant improvement in pregnancy and twinning rates. Dukelow (1967) reported the treatment of cows with WGO for 6 wk resulted in a significantly higher pregnancy rate than for controls (73 versus 43%). Silbernagel (1947) reported that consuming WGO improved fertility and prevented threatened abortion in women. However, the identity of the components producing these results was not determined.

Identification of Components in WGO and Their Potential Roles in Fruit Fly Performance. Wheat germ oil is composed of at least three saturated (myristic, palmitic, and stearic) and three unsaturated (oleic, linoleic, and linolenic) fatty acids (Nelson et al. 1963, Lercker et al. 1977, Barnes 1983, Saito and Yamauchi 1990, Saker et al. 1986) and octacosanol, a 28-carbon straight-chain saturated alcohol. The action of octacosanol is unknown. Some suggest that octacosanol may build strength and endurance in humans and affect reproduction and oxygen requirements in rats (Dukelow 1967). These results for this compound along with fatty acids coincide with our finding of increased pupal recovery, adult fliers, egg production, and egg hatch for larvae reared on WGO fortified diets. The possible roles of fatty acids in WGO that are responsible for these influences are of considerable interest. Two major polyunsaturated fatty acids of WGO are linoleic (18:2), which accounts for 42–59% of the total, and oleic (18:1), which accounts for 12 to 28% of the total (Kahlon 1989). Palmitic acid (16:0) is the major saturated fatty acid (11–19% of the total). In WGO, unsaturated fatty acids account for ≈80% and saturated ≈20% of the total fatty acid content. We speculate that fatty acids and vitamin E in WGO may be responsible for all the observed beneficial effects, because wheat germ oil is also rich in vitamin E. We have recently been investigating the incorporation of linolenic acid, linoleic acid (unsaturated fatty acids), palmitic acid (saturated fatty acids), or vitamin E into the diet individually, or in groups to determine the exact components that produce the results obtained. Preliminary tests suggest that unsaturated fatty acids (e.g., linoleic acid, oleic acid, or linolenic acid) may be responsible for lower pupal recovery, higher egg production, adult fliers, or egg hatch, whereas saturated fatty acid (e.g., palmitic acid) is responsible for lower egg hatch and higher fliers pupal recovery. Vitamin E itself may not have any effect on fruit fly performance. However, one of the functions of vitamin E is that of an antioxidant to stabilize linoleic acid, and it has an independent growth effect (Fraenkel and Blewett 1946). Our preliminary results are supported by Fraenkel and Blewett (1946) that the saponifiable fraction (linoleic acid) of wheat germ oil is necessary for emergence, growth effect, and good wing scales, and the unsapornifiable fraction (vitamin E or α-tocopherol) is necessary for proper growth. Moreover, it may be related to the nutrients contained in the

yeast. Additional studies are underway to determine the exact role(s) of these compounds.

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